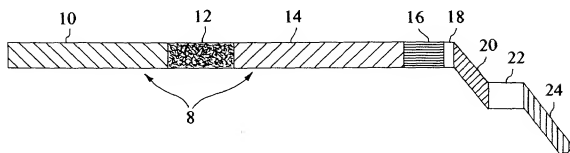
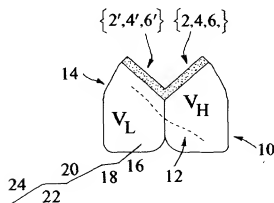


# SINGLE CHAIN BINDING POLYPEPTIDE



Extended Polypeptide

FIG. 1(a)



Folded Protein

FIG. 1(b)

SINGLE CHAIN  
BINDING POLYPEPTIDE SHOWING  
LOCATIONS OF COMPLEMENTARITY  
DETERMINING REGIONS, POLYPEPTIDE  
SPACER REGIONS, AND EFFECTOR REGIONS

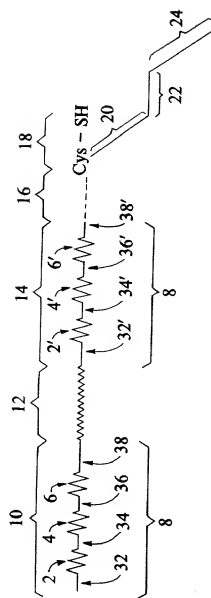


FIG. 2

C6.5 sFv  
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLQSAGELKKPGESLKISCKGYSFTSYWIAWRQMPKGLEYMGL  
IYPGSDTIKYSFQQTIVISDKVSTAYLQWSSLKPSDSVYFCARHD  
VGYSSNCAKWPFFQHWQQTGLVTWSSGGSGGGSG  
GGGQSVLTPQPSVAAPGOKVTISCSGSSNIGNNYSHVQQLPCTAPK  
LLIYHTNRPAGVDFRSGKSTSAISGFRSEDEADYYCAWDDSL  
SWTFGGTKLTVLG

FIG. 3

C6.5 sFv  
NUCLEOTIDE SEQUENCE

5'caggtgcagctgttcagctcgggacagattgaaaaacccggggagtcctcgaagatctctgaagggtctctggataca  
gctttaccagctactggatgccttgggtgcgcengatgccgggaaagcctggagtacatggggctcatctatcctggtagctc  
tgacaccaatacagcccgctcttcccaaggccaggtcaccatcctcagtcgacaaagtcgctcagcaactgcctacttgcnaatggagc  
agctcgaagccctcggacag-gccgctgtatttttgcgagacatgacgtgggatattgcagtagttcccaactgcgcaaagtggcc  
tgatacttcagcgaattggggccagggcacccttgatcacccctctctcctcaagttggagcggttcaggcggaagtggtctctggcg  
gtggcggaatcgacgtctgtgtgacgcagccctcagtgctctgggccccaggacagagagtcacacatctctctgtctctggaa  
gcagtcaccaacttgggaataattatgtacttggtaccagcagctcccagggaacagcccccaactctcatctatggtcacacca  
atcggcccg-caggggtccctgaaccgattctctfggctcccaagtctgtgacactcagctccctcgtggccatcagtggggtccggctccga  
ggatgaggctgattattactgtcagcgaatgggatgacagcctgagtggttgsggtgttcggcggaggagccaagctgacgcgtct  
agct 3'

FIG. 4

C6ML3-9 sFv'  
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAEVKPEELKISKCKGSGYSTYSLAWLWVROMPKGLEFMGL  
IYPGDSIDTKYSPFOQVTTISVDKSNVSTAYLQMSISLKPSDSAVYFCARHD  
VQYGSSSNAKWPYFQHWGQTLTVSSGGSGGGGSG  
GGGQSIVLTQPPSYSAAPQKVTISGSSSNIGNNYVSYYQLPPTAFK  
LLIYDHNRPAGVPDRFSKSGTSASLAISGRFSREADYYCASWDYTL  
SGWFGGGTKLTVLGAAAHHHHHGGGG-

FIG. 5

C6ML3-9 sFv'  
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagctcggggcagaggatgaaaagccggggagtcctcgaagatctcctgaagggtcttgata  
cagctttaccagctactggatcgctgggtgcgccagatgccgggaaaggcctggagtagacatggggctcattcctctg  
gtgactctgacaccaaataacagccgtctctcccaaggccaggtcaaccatctcagtcagtcagcaagtcctgcagcaactgcttac  
ttgcaatggagcagctcgaagccctcggacagcgctgtattttgtgcgagacatgacgtgggatatattgcagtagttc  
caactgcgaagtggtcgaatactctccagcatggggccaggccaccctggtcacccgtctctcaggtggaggcggtt  
caggcggaggtagctctggcgggtggcagctcgtgtgtgacgcgcgcctcagtgctgcggccccaggacag  
aaggtcaccatctcctcctctcgtcgtggagcagctccacacatgggaaataattatgtatctcgtgataccagcagctccccaggac  
agccccaaactctcatcatgatacaaccaatcgccgcgcagggttcctgaccgattctctggctcccaagttcggca  
cctcagctcctcggaccatcagtggggttcgggtccggatgaggtgattatctactgtcctcctgggactacacctc  
tcggcgtgggtgttcggcggaggaccaaagctgaccgtctcaggtgacggccgacaccatcatcaccatcacggctggcgg  
cggctgc 3'

FIG. 6

COML3-9 sFv'-LI-KDEL  
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVSGAEVKKPGESLKISKSGSYSTSYMIAWVRMPCKGLEYMGL  
IYFQSDTKYSPFQQTQVITSDKSVTAVTLOWESLKPSPDSANYFCARHD  
VYGVSSSNCAKPEYFQHWQCTLTIVYSGGCGSGGSGG  
GGGSQSVLTQPPSYSAAPQKVTIISCGSSSNIGNNYSWYQLPQTAPK  
LLIYDHNRPAGVDFRFGSGSKTSASLAISGRFSEADYYCASMDYTL  
SGWFGGCTKLTIVLGAHHHHHGGCGGLESSSSGSEKDEL

FIG. 7

COML3-9 sFv'-LI-KDEL  
NUCLEOTIDE SEQUENCE

5' cagggtgcagctggtgcagctcgtggggcagaggtgaaaagcccggggagctctcgaagatctcctgtaagggtctcggata  
cagctttaccagctactgagtcgctgggtggcgagatgcccggaagaaggcctggagttacatggggctcatctatcctg  
gtgactctgacacccaatatcacgcccgtctctccaaagccaggtcaccatctcagtcgacaagctcgtcagcactgcctac  
ttgcaatggagcagctcgaagccctcgacagcgcgtgtattttgtgcgagacatgacgtgggatatcgagtagttc  
caactggcgaaagtggcctgaatacttccagcattggggccagggcaacctggtcacctgtctcctcaggtggagcgggtt  
caggcgaggggtggctctggcgggtggcgagatcgcgctctgtgttgacgcagccgcctcagtgctcggccccagggacg  
aagggtcaccatctcctgctctgggaagcagctcccaacttgggaataattatgtatctcgtgtaccagcagctcccaggaac  
agcccccaactcctcatctatgacacccaatcgggcccgcaggggtccctgacacgattctctggctcccaagctcggca  
ctcagctccctcctggccatcagttgggttccggctccgagatgagctgattattactgtggcctcctgggactacaacctc  
tcgggctgggtgttcggcgaggaaccaagctgaccgtcctcaggtggcgccgcacacacatcatcaccatcaggtgggtgg  
cggctgcctcagctcctcctagctctggatccgaaaagaatgaactg 3'

FIG. 8

C6ML3-9 sFv' -L2-KDEL  
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAEVKFGESLKISKSGSYFTSYLAWROMFGKLEYMGL  
IYPGDSDTKYSPFGQGVITSYDKSYSTAYLQWSSLRFPDSAVYFCARHD  
VYCGSSNCAKPEYFOHMQCTLTIVSSGGGSGGGSGG  
CGGSQSVLTQPPSVSAAPQKVITISGSSSSNIGNVYSYQQLPGTAPK  
LLIYDITNRKFAVPRFTSGKSGTSASLASIFRSEDEADYYCASMDYTL  
SGWVFGGTKLTVLGAHHHHHGGGGCTLESSSGSSSGSEKDEL-

FIG. 9

C6ML3-9sFv' -L2-KDEL  
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagttctggggcagaggatgaaagcccggggagtcctcgaagatctctgtaagggtctctggata  
cagctttaccagctactggatcgctgggtgcccagatgcccgggaaagccctggaggtacatggggcgtcatctatcctg  
gtgactctgacaccaaatcacgcccgtctctcaagccaggtcaccatctcagtcgacaaagtcctgcagcaactgcctac  
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caactggcgaagtggcctgaatactctccagcattggggccagggcaccctggtcacctctctcaggtggaggcggtt  
caggcggaggctggctctggcggatcgagctgtgttgacgcagcgcgcctcagttgtcggccccaggaacag  
aaggtcaccatctctgctctggaaagcagctcaacattgggaataattatgtatctctggtaccagcagctcccccgaac  
agcccccaaacctctcatctatgatcacaccaatcgggccgcaggggtccctgcagcgattctctggctcccaagtctggca  
cttcagcctccctggcccatcgagtgaggctcgggtccggatcgaggagtgaaggctgattattactgtgctctgggactacacctc  
tcgggctggggtgttcggcggaggaaacaaagctgacgctcctaggtgcccgcgcacacacatcatcaccatcaggtgggtgg  
cggtcgcctcgagctagcagctcgggttctcttagctctggatccgaaaagatgaactg 3'

FIG. 10

**C6ML3-9 sFv' -L2-R14**  
**AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

—QVLVQSGAEVKKPGESLKISKGSGCYFTSYWIAWVRQMPKGLEYMGL  
 ITPGDSDTKYPFQQTIVTSVDKSVSTAYLQWSSLKTPSDSAVFCARHD  
 VQYCSNCAKWPYFQHWGQTLVTSSGGGGGGGGG  
 GGGQSULTQPPVSAAPGOKVTISCSGSSNIGNNYWYQQLCTAPK  
 LLIDYHTRPAGTPDRFSKSGKTSASLAI SGFRSEDEADYNYCASWYTL  
 SGWFGGGTKLVLYGAAAHHHHGGGGCLSSSSGSSSS  
**GSKSKAKTPKKAKP—**

**FIG. 11**

**C6ML3-9 sFv' -L2-R14**  
**NUCLEOTIDE SEQUENCE**

5' caggtcagctggtgcagctcggcgagagtgaaaaagccggsgagctctctgaagatctctctgaagggtctcggata  
 cagctttaccagctactcggatgcctgggtgcgcagatgcccgggaaagccctggagtcacatggggcctcatctatcctg  
 gtgactctgcacacaaatacagcccgctcttccaaggccaggtcaccatctcagtcgacacagtcgctcagcactgcctcac  
 ttgcaattggagcagctcgaagcctcggacagcgcgtgtattttgtgcgagacatgacgtgggataattgcagtagttc  
 caactgcgcaagtggtcctgaatacttccagcattggggccaggggcaacctggatcacgtctctctcagtgagggcgggtt  
 caggcggaggtggctctggcgggtggcggatcgtggtgacgcagccgctcagtgctgctggcggcccccaggacag  
 aaggtcacctctctcgtctgggaagcagctccacattgggaataattatgtatctctggtaccagcagctccagggaac  
 agccccaaactctctatgatcacacaaatcgcccgaggggctcctgaccgattctcttggtctcccaagttctggca  
 cctcagctctcctggccatcagtggggttccgggtccaggatgaggctgattattactgtgctcctgggactacacctc  
 tcggctgggtgtctcggcggaggaaaccaagctgcagcgtcctaggtcgggcgcacacacatcatcacatcaggtgggtgg  
 cggctgctcgagctctagcagctccgggtctcctctagctctggatcccaagaaagcgcgaaaaagaccctccgagaagaag  
 cgaagaaaccg 3'

**FIG. 12**

**C6ML3-9aFv'-L2-nls**  
**AMINO ACID SEQUENCE**

(N-terminus to C-terminus)

-QVLVSGAEVKKPGESLIKSGSYSTSYMIANVRMPKGLGYMGL  
IYFCDSDTKYSPQGVITISVDKSYSTAYLWSSIKSPDSIAVYFCARHD  
VGYCSSNCAKPEYFOHWGQGLTVTVSSGGCGCGGCGSG  
GGGSGSVLTQPPSYSAAPQKQVYIISGSSSSNIGNNYVSQYQLPCTAPK  
LLIYDHTNRPACVDFNFGSRGTSASLASGFRSEADYTCASWDYTL  
SGWVFGGKRLTVLGAAAAHHHHGGGLESSSSSSSS  
GSTPPKKKKRV

**FIG. 13**

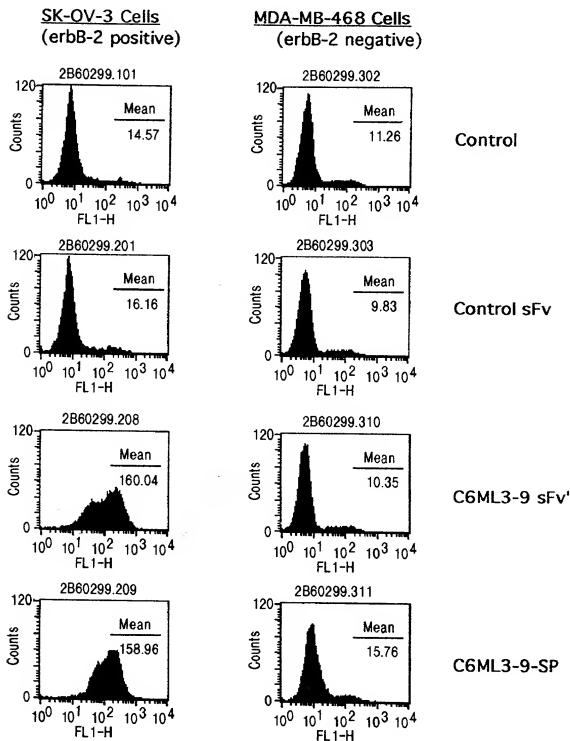
**C6ML3-9 sfv'-L2-nls**  
**NUCLEOTIDE SEQUENCE**

5' caggctgcagctggcgcagctcgtggggcagaagtgaaaaagccggggagtcctctgaagatcctcgtgaagggtctctggata  
cagcttttaacagctactggatcgcttgggtggccagatgccgggaaggccctggagatcaatggggctctatctatcctg  
gtgactctgacaccaaatacagccgctcttccaaggccaggtccaccatctcagtcgacaaagtcgctcagcactgctctac  
ttgcattggagcagctctgaagccctcggacagcgcgtgtattttgtgcgagacatgacgtgggatattgcagtagtgc  
caactgcgcgaagtggcctgaattccagcattggggccaggccacctggtaacctctctcctcaggtggagcggtt  
caggcgaggtggctctggcggatcgagctctgtgt'gacgcagccgcctctcag'tgctgcggccccaggacag  
aaggtaaccatctcctgctctggaaagcagctccaaatttgggaataattatgtatcctggtaaccagcagctccaggaaac  
agcccccnaactcttcattatgatcaccacaaatcgcccgccagggggtccctgaccgattctctggctccaangctggca  
ctcagctccctcctggcctcag'tgggtccgggtccggagatgaggtgattattactgtgcctctgggaactacaccttc  
tcgggtcgggtgttcggcggaagaaccaagatgaccgtctctaggtgcggcgacacacatcaccatcaagggtgg  
cggctcctcgagctagcagctcgggtctctctagctctcggatccactccggaaagaaacgtaaagtg 3'

**FIG. 14**



# **C6ML3-9 sFv<sup>1</sup> and its salmon protamine conjugate binds specifically to the erbB-2 positive ovarian cancer cells**



**FIG. 15**

10/18  
FACS Analysis of the erbB-2 Binding  
Activities of Bacterially Expressed C6ML3-9 sFv'  
and its Derivatives

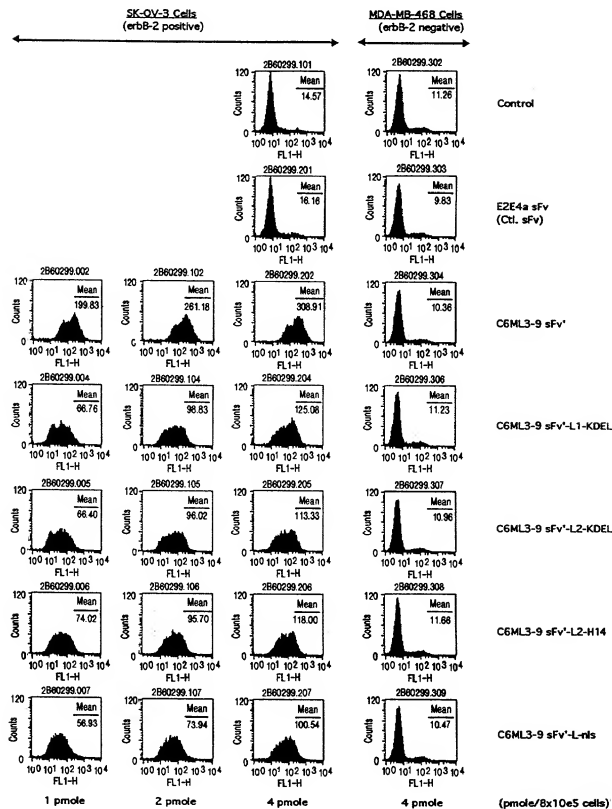
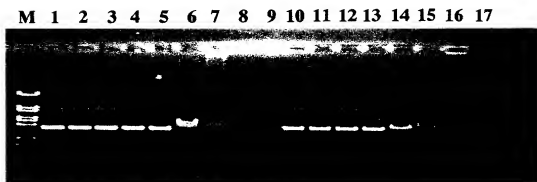


FIG. 16

0988721.030502

# Gel Shift Analysis of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex

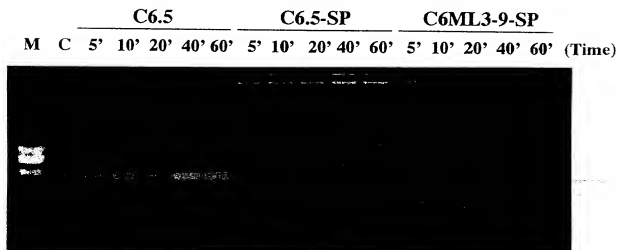


- M. DNA marker -  $\lambda$  DNA BstEII digest
1. 200 ng pGL3 DNA
  2. 200 ng pGL3 DNA + 1.45 pmol C6.5
  3. 200 ng pGL3 DNA + 2.90 pmol C6.5
  4. 200 ng pGL3 DNA + 5.80 pmol C6.5
  5. 200 ng pGL3 DNA + 11.6 pmol C6.5
  6. 200 ng pGL3 DNA + 1.45 pmol C6.5-SP
  7. 200 ng pGL3 DNA + 2.90 pmol C6.5-SP
  8. 200 ng pGL3 DNA + 5.80 pmol C6.5-SP
  9. 200 ng pGL3 DNA + 11.6 pmol C6.5-SP
  10. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9
  11. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9
  12. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9
  13. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9
  14. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9-SP
  15. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9-SP
  16. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9-SP
  17. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9-SP

\*0.8% agarose gel in 1xTAE, 150v, RT, ~1hr, EtBr staining overnight

FIG. 17

# **Kinetic Study of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex Formation**



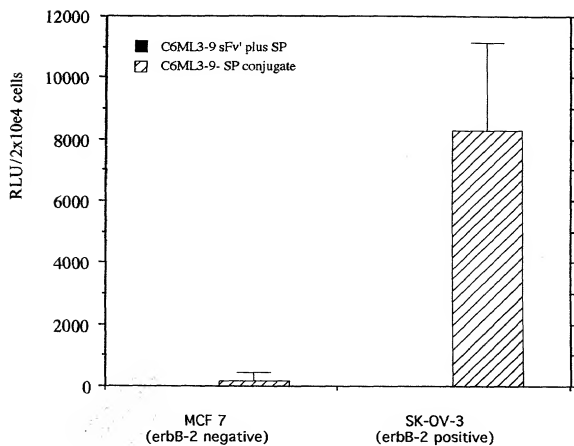
M. DNA marker -  $\lambda$  DNA BstEII digest

C. 200 ng pGL3 DNA alone

\* The rest of the lanes - 200 ng pGL3 DNA incubated with 5.8 pmol proteins as indicated above each line, on ice, for different period of time. Electrophoresis condition same as Figure 17.

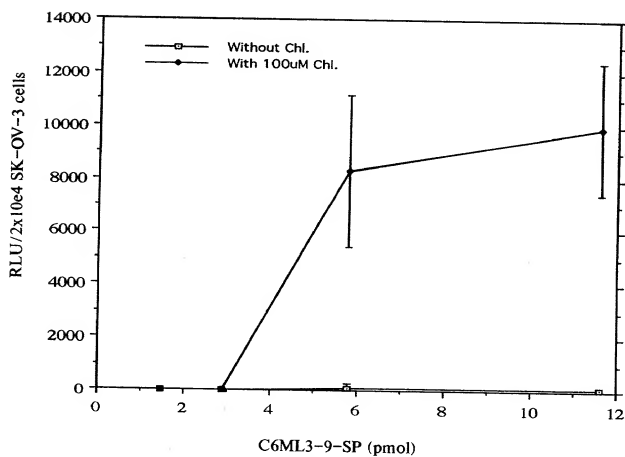
**FIG. 18**

**The C6ML3-9-SP conjugate protein mediates  
specific luciferase gene delivery to erbB-2 positive cancer cells**



**FIG. 19**

### Chloroquine-dependent C6ML3-9-SP-mediated Gene Delivery

**FIG. 20**

Fluorescent microscopy of C6.5-SP and C6ML3-9  
-SP-mediated gene transfer of pGeneGrip Rhodamine/GFP  
plasmids with SK-OV-3 and MCF-7

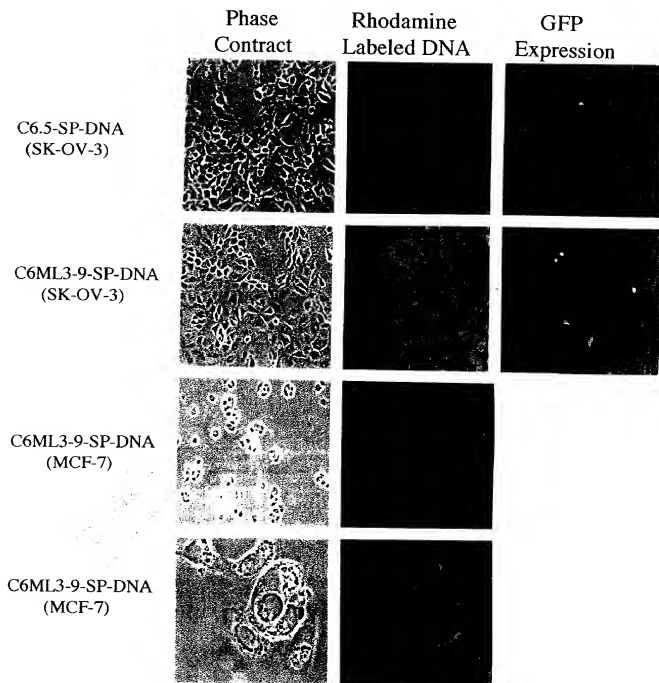


FIG. 21

THE EFFECT OF  
CHLOROQUINE ON 3T3-HER2 TRANSFECTION  
MEDIATED BY C6ML3-9sFv'-SALMON PROTAMINE

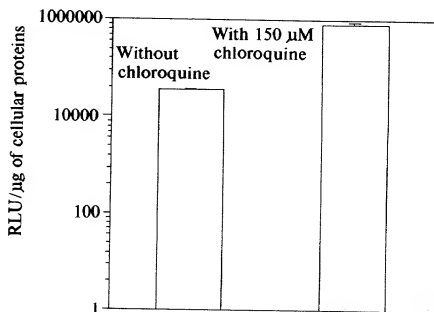


FIG. 22

THE EFFECT OF CHLOROQUINE ON 3T3-HER2  
TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-P1

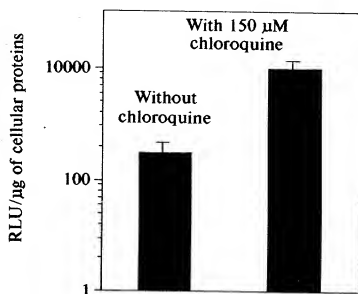


FIG. 23



17/18

# THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

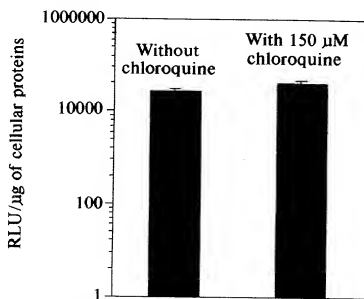


FIG. 24

# THE EFFECT OF C6ML3-9sFv'-H1-pBks ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

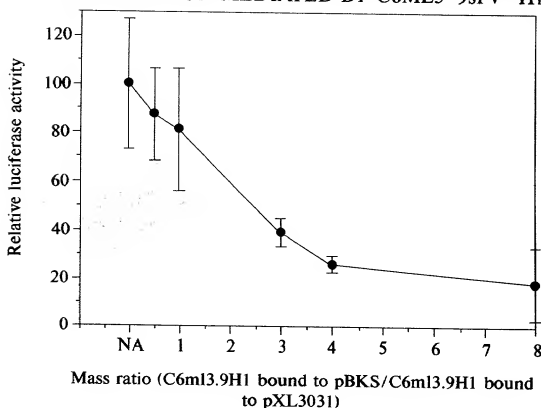


FIG. 25

THE EFFECT OF THE DNA TO C6ML3-9sFv'-H1  
RATIO ON 3T3-HER2 TRANSFECTION EFFICIENCY

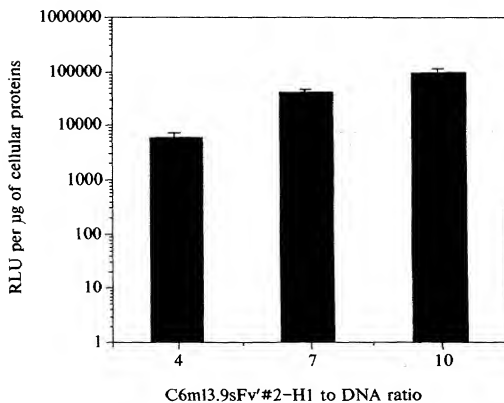


FIG. 26